

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
Page 4

This listing of claims will replace all prior versions, and listings, of claims in the application.

In the Claims:

Claims 1-35 (canceled)

B2  
Claim 36 (currently amended): An isolated or recombinantly expressed antifreeze protein, said protein comprising the following:

- (i) a calculated molecular weight of between 7 and 13 kDa;
- (ii) a thermal hysteresis activity of greater than ~~about~~ 1.5°C at a concentration of about 1 mg/mL;
- (iii) the N-terminal amino acid motif set forth in SEQ ID NO:3;
- (iv) specific binding to an antibody raised against an antifreeze protein ~~selected from the group consisting of YL-1 (SEQ ID NO:11), YL-2 (SEQ ID NO:13), YL-3 (SEQ ID NO:17), and YL-4 (SEQ ID NO:15);~~ and
- (v) at least ~~about~~ 70% amino acid sequence identity to an antifreeze protein ~~selected from the group consisting of YL-1 (SEQ ID NO:11), YL-2 (SEQ ID NO:13), YL-3 (SEQ ID NO:17), and YL-~~

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
Page 5

~~4 (SEQ ID NO:15).~~

Claim 37 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein comprises at least one repeat of the 12 contiguous amino acid motif set forth in SEQ ID NO:1.

Claim 38 (original): The isolated or recombinant antifreeze protein of claim 37, wherein the number of repeats of the motif is from 5 to 12.

Claim 39 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the calculated molecular weight of the antifreeze protein is between 8 and 12 kDa.

Claim 40 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein includes the subsequence of amino acids set forth in SEQ ID NO:4.

---

B3 Claim 41 (currently amended): The isolated or recombinant antifreeze protein of claim 36, wherein the thermal hysteresis activity is greater than ~~about~~ 2°C at a concentration of about 1

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
Page 6

mg/mL.

*Out*  
*B3*  
Claim 42 (currently amended): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is ~~selected from the group consisting of YL-1 (SEQ ID NO:11), YL-2 (SEQ ID NO:13), YL-3 (SEQ ID NO:17), and YL-4 (SEQ ID NO:15).~~

---

Claim 43 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is expressed by a baculovirus vector.

Claim 44 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is synthesized by a bacterial cell, a fungus cell, a plant cell, or an animal cell.

Claim 45 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is synthesized by a yeast cell.

Claim 46 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
Page 7

synthesized by an animal cell.

Claim 47 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the nucleic acid encoding the antifreeze protein is synthesized by an insect cell.

Claim 48 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is derived from *Tenebrio* sp.

Claim 49 (original): The isolated or recombinant antifreeze protein of claim 44, wherein the antifreeze protein is expressed externally from the cell.

Claims 50-77 (canceled)

---

by Claim 78 (currently amended): A liquid comprising a recombinant antifreeze protein, said antifreeze protein comprising the following:

- (i) a calculated molecular weight of between 7 and 13 kDa;
- (ii) a thermal hysteresis activity of greater than ~~about~~ 1.5°C at a concentration of about 1 mg/mL;

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
Page 8

Cont  
By  
(iii) the N-terminal amino acid motif set forth in SEQ ID  
NO:3;

(iv) specific binding to an antibody raised against an  
antifreeze protein ~~selected from the group consisting of YL-1~~  
~~(SEQ ID NO:11), YL-2 (SEQ ID NO:13), YL-3 (SEQ ID NO:17), and YL-~~  
~~4 (SEQ ID NO:15); and~~

(v) at least ~~about~~ 70% amino acid sequence identity to an  
antifreeze protein ~~selected from the group consisting of YL-1~~  
~~(SEQ ID NO:11), YL-2 (SEQ ID NO:13), YL-3 (SEQ ID NO:17), and YL-~~  
~~4 (SEQ ID NO:15).~~

---

Claim 79 (original): The liquid of claim 78, wherein the  
antifreeze protein comprises at least one repeat of the 12  
contiguous amino acid motif set forth in SEQ ID NO:1.

Claim 80 (original): The liquid of claim 78, wherein the  
concentration of antifreeze protein is between about one part per  
billion (1 µg/L) to about one part per thousand (1 g/L).

B5  
Claim 81 (currently amended): The liquid of claim 78, wherein  
the thermal hysteresis activity is greater than ~~about~~ 2°C at a  
concentration of about 1 mg/mL.

---